



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference A-147228	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/ES99/00378	International filing date (day/month/year) 24/11/1999	Priority date (day/month/year) 25/11/1998
International Patent Classification (IPC) or national classification and IPC C07K14/815		
Applicant UNIVERSITAT AUTONOMA DE BARCELONA et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 4 sheets, including this cover sheet.

This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

- I Basis of the report
- II Priority
- III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV Lack of unity of invention
- V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI Certain documents cited
- VII Certain defects in the international application
- VIII Certain observations on the international application

Date of submission of the demand 19/06/2000	Date of completion of this report 16.03.2001
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Fotaki, M Telephone No. +49 89 2399 8709



INTERNATIONAL PRELIMINARY
EXAMINATION REPORT

International application No. PCT/ES99/00378

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17.)*):

Description, pages:

1-15 as originally filed

Claims, No.:

1-12 as received on 21/02/2001 with letter of 19/02/2001

Sequence listing part of the description, pages:

1,2, filed with the letter of 6.1.00

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- the language of publication of the international application (under Rule 48.3(b)).
- the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- contained in the international application in written form.
- filed together with the international application in computer readable form.
- furnished subsequently to this Authority in written form.
- furnished subsequently to this Authority in computer readable form.
- The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- the description, pages:
- the claims, Nos.:
- the drawings, sheets:

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT**

International application No. PCT/ES99/00378

5. This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

II. Priority

1. This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:

copy of the earlier application whose priority has been claimed.

translation of the earlier application whose priority has been claimed.

2. This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N) Yes: Claims 1-12
 No: Claims

Inventive step (IS) Yes: Claims 1-12
 No: Claims

Industrial applicability (IA) Yes: Claims 1-12
 No: Claims

2. Citations and explanations
see separate sheet

INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET

International application No. PCT/ES99/00378

II. PRIORITY

- 1) This international preliminary examination report has been established after consideration of the priority document ES 9802524 of 25.11.98. Therefore, document REVERTER D ET AL: 'A Carboxypeptidase Inhibitor from the Medical Leech *Hirudo medicinalis*' The Journal of Biological Chemistry, vol. 273, num. 49, 1998, p. 32927-32933 cited in the International Search Report are not relevant in establishing the novelty of the present invention.

V. REASONED STATEMENT UNDER ARTICLE 35(2)

- 2) The present application relates to the identification of a *Hirudo medicinalis*-derived protein and the encoding nucleotide sequence (SEQ ID NO 1) which is an inhibitor of metallocarboxypeptidase B, designated Leech Carboxypeptidase inhibitor (LCI). The latter is an inhibitor of plasminogen-activated fibrinolysis and thus, the identified protein is a fibrinolytic agent. The presence of the identified protein was demonstrated to result in faster lysis of fibrin clot in vitro.

In light of the cited prior art documents, the identification of such a protein was not disclosed nor obvious and thus, the subject-matter of **Claims 1-13** is novel and inventive as required by Article 33 PCT.

CLAIMS

1. A recombinant nucleotide sequence identified as SEQ ID 1 that encodes a protein sequence corresponding to a 5 metallocarboxypeptidase inhibitor from *Hirudo medicinalis*.
2. A polypeptide sequence encoded by the nucleotide sequence according to claim 1, characterized in that it comprises the sequence identified as SEQ ID N° 2 of the list of sequences.
- 10 3. A polypeptide sequence according to claim 2, wherein such sequence is homologous to the sequence identified as SEQ N° 2.
4. A nucleotide sequence that comprises a coding sequence of a polypeptide homologous to the sequence ID N° 2 15 according to claim 2.
5. A prokaryotic or eukaryotic expression vector characterized in that it includes the recombinant nucleotide sequence of any of claims 1 or 4, and in that it is able to express the biologically active 20 metallocarboxypeptidase inhibitor.
6. A transformed *Escherichia coli* cell characterized in that it comprises an expression vector according to claim 5 and in that it is able to produce the biologically active metallocarboxypeptidase inhibitor.
- 25 7. A procedure to prepare a recombinant metallocarboxypeptidase inhibitor identified as SEQ ID 2 according to any of claims 2 to 3 characterized in that it comprises
 - (i) the culture of the transformant that contains 30 an expression vector capable of expressing a biologically active metallocarboxypeptidase inhibitor; and
 - (ii) its obtention and purification.
8. A procedure according to claim 7 characterized in that the recombinant process takes place in a prokaryotic or 35 eukaryotic host.

REPLACED BY
ART 34 AMDT

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9. A metallocarboxypeptidase inhibitor according to claims 2 or 3, as fibrinolytic agent.
10. Use of the metallocarboxypeptidase inhibitor according to claims 2 or 3, to prepare a drug useful as fibrinolytic agent.
11. Use of the metallocarboxypeptidase inhibitor according to claim 10, in combination with other fibrinolytic agents which it complements or enhances, to prepare a drug useful as fibrinolytic agent.
- 10 12. A pharmaceutical composition that comprises, as active agent, an effective quantity of a metallocarboxypeptidase inhibitor identified as SEQ ID 2, or its derivatives, and a pharmaceutically acceptable excipient.